Environmental Health Perspectives
Vol. 88, pp. 5–12, 1990

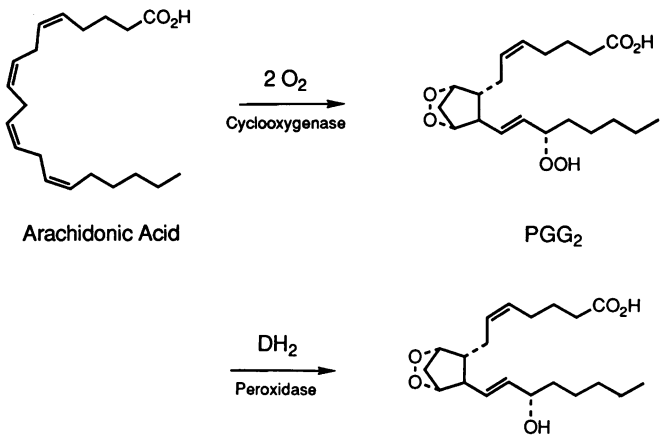
Prostaglandin Synthase-Mediated Metabolism of Carcinogens and a Potential Role for Peroxyl Radicals As Reactive Intermediates

by Lawrence J. Marnett*

Prostaglandin-H synthase is unique among enzymes of the plant and animal kingdom in its ability to biosynthesize and metabolize hydroperoxides. Its cyclooxygenase activity oxygenates polyunsaturated fatty acids to hydroperoxy endoperoxides, and its peroxidase activity reduces the hydroperoxy group to hydroxy groups. Higher oxidation states of the peroxidase oxidize reducing substrates to electron-deficient derivatives that react with macromolecular nucleophiles. In the case of aromatic amines, the electron-deficient derivatives are mutagenic to bacterial and mammalian cells. β-Dicarbonyl compounds and retinoic acid are oxidized to carbon-centered radicals that react with O_2 to form peroxyl free radicals. Peroxyl radicals are the most stable oxy radicals and are able to diffuse some distance from the site of their generation. Peroxyl radicals are also formed during lipid peroxidation and in the reaction of polyunsaturated fatty acid hydroperoxides with metal complexes and metalloproteins. Peroxyl radicals epoxidize isolated doubled bonds of compounds such as 7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene (BP-7,8-diol); 3,4-dihydroxy-3,4-dihydrobenzo(*a*)anthracene; and aflatoxin B_1. The epoxide products represent the ultimate carcinogenic forms of the respective compounds. Techniques for quantitating the extent of peroxidase dependent or peroxyl radical-dependent metabolism *in vivo* make use of differences in the structure or stereochemistry of reactive intermediates formed by peroxidases relative to cytochromes P-450. Differences in the relative amounts of hydrolysis products and DNA adducts derived from *anti*- and *syn*-dihydrodiolepoxides following application of BP-7,8-diol to mouse skin *in vivo* indicate peroxyl radicals play a significant role in metabolism of BP-7,8-diol in uninduced animals.

Introduction

Prostaglandin-H (PGH) synthase catalyzes the oxygenation of arachidonic acid to the hydroperoxy endoperoxide, PGG_2, and the reduction of PGG_2 to the hydroxy endoperoxide, PGH_2 [Eq. (1)] (*1–3*). PGH synthase is unique among all plant and animal peroxidases because it has the ability to biosynthesize the hydroperoxide substrate for its peroxidase activity. There has been a great deal of interest in the possible involvement of PGH synthase in the oxidative metabolism of drugs and carcinogens (*4–7*). The tissue distribution and mechanisms of oxidation of PGH synthase are distinct from those exhibited by the principal drug-metabolizing enzyme in animal tissue, cytochrome P-450. This raises the possibility that PGH synthase serves as an alternative metabolic activation enzyme to the P-450 isoenzymes. There appears little chance that PGH synthase plays a major role in systemic drug metabolism; but it may be involved in tissue-specific pathology by certain toxins or carcinogens. For example, PGH synthase-dependent oxidation is implicated in bladder carcinogenesis by aromatic amines and nitrofurans and in teratogenicity by trimethadone (*7,8*).



(1)

*Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232.

Properties of PGH Synthase

Other mammalian peroxidases carry out similar oxidations to PGH synthase. Why, then, is there special interest in this particular peroxidase? Figure 1 illustrates some of the influences on PGH synthase that provide a partial answer to this question. The profile of polyunsaturated fatty acids in phospholipids and triglycerides is sensitive to the makeup of the fat eaten by particular individuals. Thus, there are dietary influences on the substrates that PGH synthase oxidizes. The cyclooxygenase activity of PGH synthase only oxidizes free fatty acids, the concentrations of which are very low in most cells (*9,10*). Release of substrate is the limiting step in prostaglandin biosynthesis and is triggered by the interaction of membrane-active agents with the cell surface (*11*). This makes PGH synthase and the oxidations it catalyzes exquisitely sensitive to events at the plasma membrane. The oxygenation of arachidonic acid by PGH synthase is potently inhibited by a variety of nonsteroidal anti-inflammatory drugs. In fact, it is generally believed that this inhibition explains the mechanism of anti-inflammatory activity of such drugs. Aspirin is noteworthy among the well-known anti-inflammatory agents because it inhibits PGH synthase by selectively acetylating the hydroxyl group of Ser^{506} (*12*).

Recent work establishes that the levels of PGH synthase in cells are sensitive to stimulation at the transcriptional and translational level by a variety of growth factors and tumor promoters, including epidermal growth factor (EGF), transforming growth factor β, interleukin-1 (IL-1), tetradecanoyl phorbol acetate, and tumor necrosis factor (*13–17*). Enhanced expression of the PGH synthase gene by EGF and IL-1 appear to be inhibited by corticosteroids (*18,19*), which may explain the potent anti-inflammatory activity of EGF and IL-1. An alternative mechanism for rapid modulation of PGH synthase protein levels is supported by the report that the half-life of the protein in rat muscle is less than 10 min (*20*). This half-life implies rapid turnover by proteolysis, but the proteases responsible and the generality of their action in other tissues has not been established. The summation of these observation identifies PGH synthase as an enzyme that triggers intracellular oxidations in response to events at the cell surface while demonstrating sensitivity to a variety of physiological and pharmacological modulators.

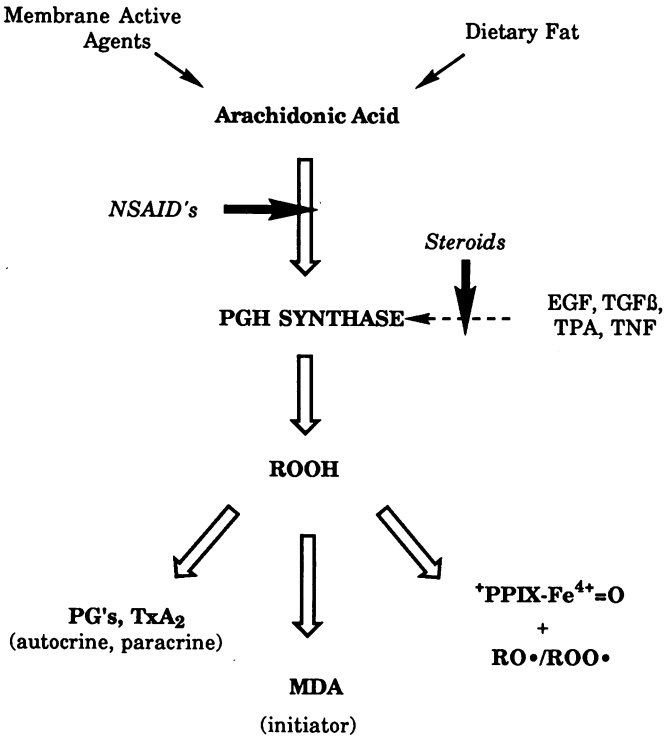


FIGURE 1. Modulation of arachidonic acid metabolism and PGH synthase. See text for details.

The initial product of PGH synthase action is the hydroperoxy endoperoxide PGG_2. Its hydroperoxide group triggers the formation of potent oxidizing agents, either metal-oxo derivatives of the heme prosthetic group or oxygen radicals derived from the hydroperoxide (*21,22*). The endoperoxide group is metabolized to prostaglandins and thromboxane, which are potent autocrine and paracrine mediators of cell responses. PGG_2 is also converted to malondialdehyde, which is a mutagen and carcinogen (*23–25*). Although the emphasis of this paper is on mechanisms of oxidation by PGH synthase, which is usually associated with tumor initiation, PGH synthase may also play an important role in proliferative events such as those associated with tumor promotion and progression (*26*).

PGH synthase is widely distributed in mammalian tissue but is particularly abundant in accessory sex glands, lung, and vasculature (*27*). PGH synthase is a membrane protein located in the endoplasmic reticulum, the nuclear membrane, and occasionally in the plasma membrane (*28,29*). The enzyme purified from ovine or bovine seminal vesicles is a homodimer of 70 kDa subunits (*30–38*). It contains 3.5% carbohydrate of the high mannose type and variable amounts of ferric protoporphyrin IX (Fe^{3+}-PPIX) (*39*). The heme group is essential for both cyclooxygenase and peroxidase activities but is readily lost during solubilization and chromatography. Both activities are reconstituted following addition of Fe^{3+}-PPIX, whereas reconstitution with Mn^{3+}-PPIX produces an enzyme with high cyclooxygenase but low peroxidase activity (*40*). There is some disagreement about the stoichiometry of heme binding, but most estimates place it between one and two hemes per homodimer (*41,42*). The amino acid sequence of PGH synthase is available from its cDNA sequence (*43–45*). The open reading frame codes for 600 amino acids, of which the first 24 represent a signal sequence that is absent in the mature protein. Computer analysis reveals no striking parallels to other functionally related proteins. Speculation has been offered for the location of membrane binding and heme binding regions of the polypeptide (*43,46*).

Hydroperoxide-Dependent Oxidations

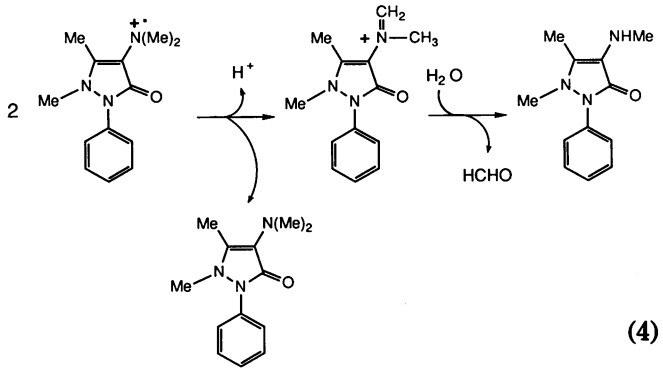
Cooxidation during arachidonic acid oxygenation results from interactions of PGG_2 or other hydroperoxy fatty acids with the heme prosthetic group of PGH synthase or other metals and metalloproteins in the cells. The two general pathways of hydroperoxide reduction by metal complexes are shown in Equations (2) and (3). One-electron reduction of the hydroperoxide produces an alkoxyl free radical and a ferryl-oxo complex that is one redox equivalent above Fe^{3+}-PPIX [Eq. (2)]. Two-electron reduction of the hydroperoxide produces an alkoxide ion and a ferryl-oxo complex that is two redox equivalents above Fe^{3+}-PPIX [Eq. (3)]. Both ferryl-oxo complexes and the alkoxyl free radical are potent oxidizing agents. Studies with a diagnostic hydroperoxide probe, 10-hydroperoxy-octadeca-8,12,-dienoic acid, establish that the heme prosthetic group of PGH synthase reduces hydroperoxides by two electrons [Eq. (3)] (*46*). The two-electron oxidized heme has been detected by rapid scan spectrophotometry and appears electronically very similar to the higher oxidation state known as compound I of horseradish peroxidase (*47,48*). The prosthetic group of compound I contains a ferryl-oxo complex (Fe^{4+}-O) and a porphyrin cation radical (*49*).

$$ROOH + M^{n+} \rightarrow RO^{\cdot} + M^{n+1} \quad (2)$$

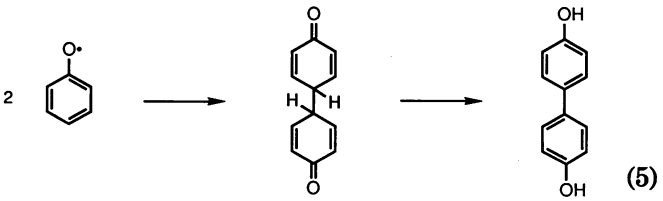
$$ROOH + M^{n+} \rightarrow ROH + M^{n+2} \quad (3)$$

PGH synthase catalyzes a number of oxidations that appear similar to those of horseradish peroxidase, but a comprehensive comparison of the substrate specificity of the two enzymes has not been performed. All the available mechanistic information indicates PGH synthase follows a classic peroxidase catalytic cycle (Fig. 2). Reduction of the hydroperoxide oxidizes the heme to compound I. This intermediate must be reduced by two electrons to regenerate resting enzyme. Heme reduction occurs by either stepwise electron transfer or what appears to be concerted oxygen transfer. Oxygen transfer is only observed with alkyl aryl sulfides (*50,51*).

Certain amines and phenols are excellent substrates for electron transfer oxidation by peroxidases. Amines produce cation radicals and phenols produce phenoxyl radicals (*52*). Certain β-dicarbonyl compounds and polyenes are oxidized to carbon-centered radicals by electron transfer or hydrogen atom abstraction (*53–55*). The fate of the one-electron oxidized derivatives of the reductants determines the eventual products of the reaction; for example, amine cation radicals disproportionate to iminium cations and the parent amine [Eq. (4)] (*56*). The iminium compounds undergo subsequent reactions such as hydrolysis. Phenoxyl radicals couple to form biphenols that can be further oxidized to quinonoid compounds [Eq. (5)] (*52*). Diamines and polyphenolic compounds (benzidine, catechol) are particularly good substrates and are oxidized to quinoneimines and quinones, respectively (*6,57,58*). Some of the one-electron oxidized derivatives of the reducing substrates or their metabolites (e.g., amine cation radicals, quinoneimines, and quinones) react with nucleophilic sites on proteins and nucleic acids. This reaction has been suggested to account for the toxicity/mutagenicity or transforming activity of aromatic amines, nitrofurans, and diethylstilbestrol (*7,59–61*).



(4)



(5)

Peroxyl Free Radicals

In contrast to the reactions of nitrogen- and oxygen-centered radicals, carbon-centered radicals couple to molecular oxygen to form peroxyl radicals (*62*). This is a very important reaction because peroxyl radicals are long-lived and capable of epoxidation of isolated double

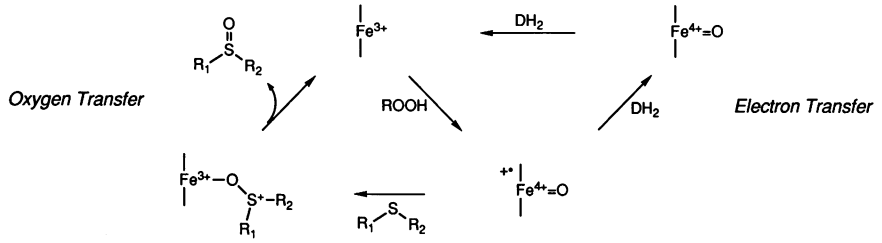
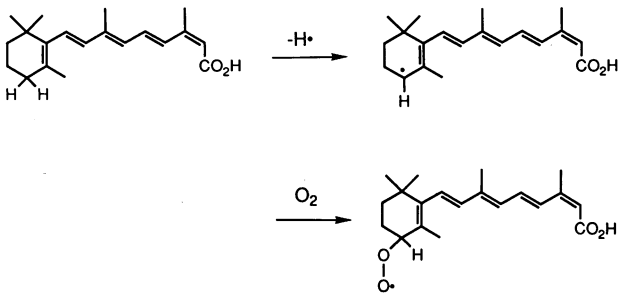
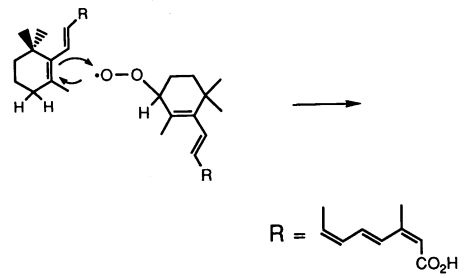


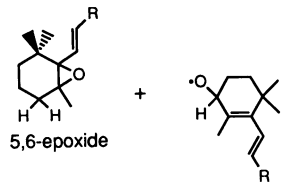
FIGURE 2. Peroxidase catalytic cycle.

bonds. A particularly interesting example of this epoxidation is provided by retinoic acid. Retinoic acid is a potent inhibitor of tumor promotion in mouse skin (*63–65*) and is a moderately good reducing substrate for the peroxidase of PGH synthase (*54*). Retinoic acid is oxidized to a carbon-centered radical that forms a peroxyl radical [Eq. (6)]. This peroxyl radical then epoxidizes another molecule of retinoic acid, producing equal amounts of 4-hydroxy retinoic acid and 5,6-epoxy retinoic acid [Eq. (7)] (*54*). The reactivity of retinoic acid toward peroxyl radicals is quite high among a series of polyenes we have tested. This makes retinoic acid an excellent radical scavenger and antioxidant (*66*). Whether this property contributes to its chemopreventive activity is unknown.



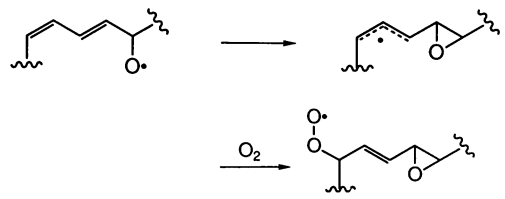
(6)





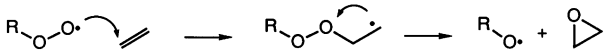
(7)

Another way to make carbon radicals and, therefore, peroxyl radicals, is the cycling of polyunsatured alkoxyl radicals [Eq. (8)] (*67*). Alkoxyl radicals produced by one-electron reduction of polyunsatured fatty acid hydroperoxides are transformed to peroxyl radicals in moderate yield, and this accounts for a significant proportion of hydroperoxide-dependent oxidations catalyzed by metal complexes and metalloproteins (*68*).



(8)

Peroxyl radicals are recognized as the principal mediators of lipid peroxidation, but they have been essentially overlooked as mediators of hydroperoxide-dependent oxidations until recently (*69,70*). In contrast to alkoxyl radicals and metal-oxo complexes, peroxyl radicals are relatively stable and somewhat selective in their reactions. The half-lives of peroxyl radicals range from 0.1 to 10 sec in the biological mileu (*71*). This half-life means that peroxyl radicals are able to diffuse to cellular locations remote from the site of their generation to effect oxidations. An important reaction of peroxyl radicals is their ability to epoxidize olefins [Eq. (9)] (*72*). It is not certain what endogenous cellular compounds, if any, are epoxidized, but there is no doubt xenobiotics and their metabolites are epoxidized by peroxyl radicals (*70*).



(9)

In addition to the anticarcinogen retinoic acid, peroxyl radicals epoxidize the procarcinogens 7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene (BP-7,8-diol); 3,4-dihydroxy-3,4-dihydrobenzo(*a*)anthracene; and aflatoxin B_1 (*73–77*). The epoxide products of each of these reactions represent the ultimate carcinogenic forms of the parent molecules [benzo(*a*)pyrene and benzo(*a*)anthracene for the first two compounds]. Peroxyl radical-dependent epoxidation of BP-7,8-diol has been detected during such diverse processes as PGH synthase-dependent cooxidation, NADPH-dependent lipid peroxidation, cumene hydroperoxide-dependent-lipid peroxidation, hematin-catalyzed decomposition of polyunsaturated fatty acid hydroperoxides, peroxidase-dependent oxidation of β-dicarbonyl compounds, and peroxidase-dependent oxidation of bisulfite (*74,76,78–81*). The common thread through these reactions is the generation of peroxyl free radicals.

Techniques for Quantitating Hydroperoxide-Dependent Oxidations

Quantitating the extent to which hydroperoxide-dependent xenobiotic oxidations occur in intact cells or *in vivo* requires specific methodology for each compound of interest. In some cases, one can exploit the different mechanisms of oxidation of xenobiotics by peroxidases compared to cytochrome P-450 to design experiments to detect peroxidase products. For example, aromatic

monoamines are oxidized by peroxidases to radical cations that form different adducts to DNA than do the nitrenium ions formed in cytochrome P-450-dependent reactions (*82*). Peroxidase-dependent adducts have been detected in DNA from the urinary bladders of dogs fed β-naphthylamine (*83*). Unfortunately, the identities of the adducts have not been elucidated, and their formation appears quite variable. Thus, the generality of the formation of the adducts is not certain.

Stereochemistry has been used to differentiate epoxidation of polycyclic hydrocarbon dihydrodiols by peroxyl radicals and cytochromes P-450 (*84*). Figure 3 summarizes the stereochemical considerations that make this approach feasible. The (−)-enantiomer of BP-7,8-diol is epoxidized to the (+)-enantiomer of the *anti*-dihydrodiolepoxide by both peroxyl radicals and cytochromes P-450. In contrast, the (+)-enantiomer of BP-7,8-diol is epoxidized to the (−)-enantiomer of the *anti*-dihydrodiolepoxide by peroxyl radicals, but to the (+)-enantiomer of the *syn*-dihydrodiolepoxide by cytochromes P-450 (*84*). Dihydrodiolepoxides hydrolyze to tetrahydrotetraols that are readily separable by HPLC, which enables one to quantitate the amounts of the dihydrodiolepoxides produced and to identify the oxidant responsible for their formation (*85*). Alternatively, one can isolate DNA modified by BP-7,8-diol oxidation products, hydrolyze it to deoxynucleoside adducts, and quantitative the levels of adducts produced by reaction with the various dihydrodiolepoxide enantiomers and diastereomers. By starting with commercially available (+)-BP-7,8-diol, one can directly determine the percentage of epoxides produced by peroxyl radicals or cytochrome P-450.

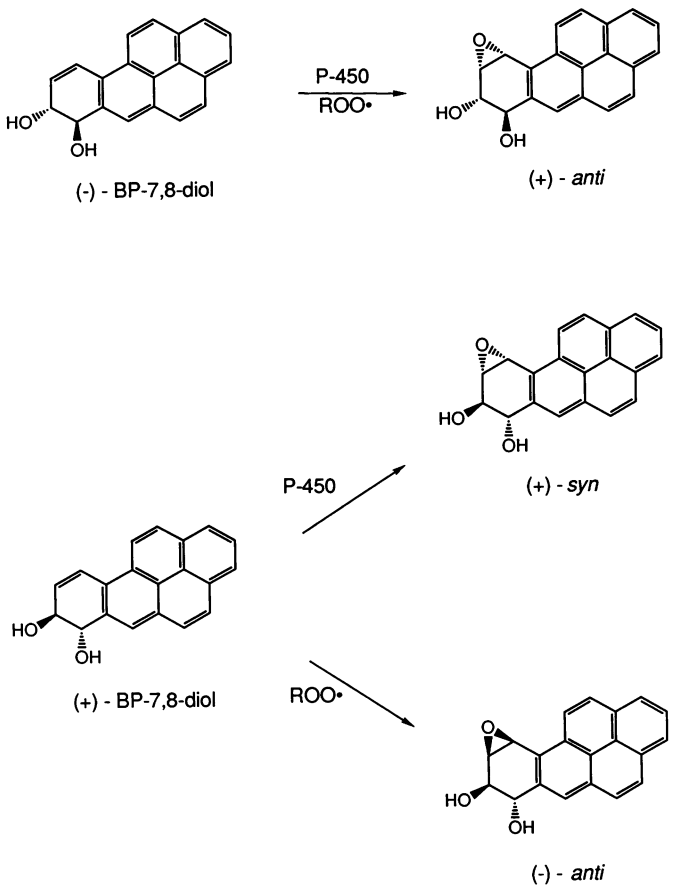


FIGURE 3. Comparison of the stereochemistry of epoxidation of BP-7,8-diol by cytochrome P-450 and peroxyl radicals.

The stereochemical approach has been employed to detect peroxyl radical-dependent epoxidation of BP-7,8-diol in rat liver microsomes, mouse skin homogenates, cultured fibroblasts, cultured hamster trachea, and freshly isolated mouse epidermal cells (*79,86–89*). Figure 4 displays HPLC profiles of the products of (+)-BP-7,8-diol oxidation by epidermal homogenates from control female CD-1 mice and mice pretreated with the cytochrome P-450 inducer β-naphthoflavone (*86*). Peroxyl radical products predominate in the uninduced animals, whereas cytochrome P-450 products are the principal metabolites in β-naphthoflavone-pretreated animals. This suggests that peroxyl radicals are important contributors to BP-7,8-diol oxidation in the skin of normal animals, whereas cytochrome P-450 has primacy in induced animals. Since BP-7,8-diol is a tumor initiator in mouse skin but is not a cytochrome P-450 inducer, it seems peroxyl radicals play a significant role in the metabolic activation of BP-7,8-diol to an ultimate carcinogenic form in at least one tissue commonly used to assay tumor initiators (*86,89*). These conclusions are sup-

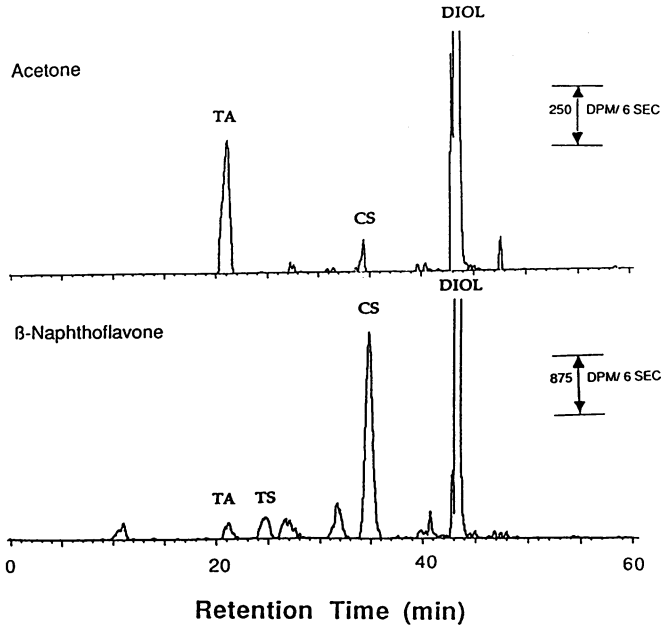


FIGURE 4. Reverse-phase HPLC profiles of the tetraol hydrolysis products formed after (+)-BP-7,8-diol epoxidation by mouse skin homogenates. (TA) *trans-anti*-tetraol, the major hydrolysis product of the (−)-*anti*-dihydrodiolepoxide; (CS) *cis-syn*-tetraol, the major hydrolysis product of the (+)-*syn*-dihydrodiolepoxide; and (TS) *trans-syn* tetraol, the minor hydrolysis product of the (+)-*syn*-dihydrodiolepoxide. Reprinted with permission (*86*).

ported by studies of BP-7,8-diol metabolism to tetraols or DNA adducts in mouse skin *in vivo* (*86,90*).

A common approach to detecting metabolic pathways is the use of specific inhibitors. Numerous compounds inhibit the cyclooxygenase activity of PGH synthase (*91*). These compounds do not block the peroxidase activity of the enzyme, but by preventing PGG_2 synthesis, they inhibit PGH synthase-dependent cooxidation. Agents such as aspirin and indomethacin are quite useful in determining the percentage oxidation of a particular compound dependent on PGG_2 biosynthesis. However, PGH synthase inhibitors do not block hydroperoxide synthesis by lipoxygenases or by lipid peroxidation. This fact makes PGH synthase inhibitors of limited utility for diagnosing the overall contribution of hydroperoxide-dependent oxidation to the metabolism of a given compound, particularly in intact cells or *in vivo*. For example, indomethacin does not inhibit oxidation of (+)-BP-7,8-diol to (−)-*anti*-dihydrodiolepoxide by freshly isolated mouse epidermal cells (*89*). Other biochemical studies establish that the peroxides responsible for BP-7,8-diol oxidation in epidermis arise from lipid peroxidation (*89*).

Even if one does see inhibition of oxidation of a compound by an inhibitor of PGH synthase, one must interpret the observation with caution. In intact cells, toxicity or alteration of cell cycle kinetics can be depress the extent of metabolism and/or DNA adduction without actually inhibiting the oxidative reaction. Conclusions about the role of PGH synthase cooxidation in xenobiotic metabolism based on inhibitor studies should be supported by parallel studies of the effects of the inhibitors on the metabolites and macromolecular adducts derived from the test compounds.

Work on this project has been supported over the years by research grants from the American Cancer Society (BC-244 and FRA-243) and the National Institutes of Health (GM 23642, CA 43209, CA 47479).

REFERENCES

1. Ohki, S., Ogino, N., Yamamoto, S., and Hayaishi, O. Prostaglandin hydroperoxidase, an integral part of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. J. Biol. Chem. 254: 829–836 (1979).
2. Hamberg, M., Svensson, J., Wakabayashi, T., and Samuelsson, B. Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. Proc. Natl. Acad. Sci. USA 71: 345–349 (1974).
3. Nugteren, D. H., and Hazelhof, E. Isolation and properties of intermediates in prostaglandin biosynthesis. Biochem. Biophys. Acta 326: 448–461 (1973).
4. Marnett, L. J. Arachidonic acid metabolism and tumor initiation. In: Arachidonic Acid Metabolism and Tumor Initiation (L. J. Marnett, Ed.), Martinus Nijhoff, Boston, 1985, pp. 39–82.
5. Eling, T. E., and Krauss, R. S. Arachidonic acid-dependent metabolism of chemical carcinogens and toxicants. In: Arachidonic Acid Metabolism and Tumor Initiation (L. J. Marnett, Ed.), Martinus Nijhoff, Boston, 1985, pp. 83–124.
6. Markey, C. M., Alward, A., Weller, P. E., and Marnett, L. J. Quantitative studies of hydroperoxide reduction by prostaglandin H synthase. J. Biol. Chem. 262: 6266–6279 (1987).
7. Rice, J. R., Zenser, T. V., and Davis, B. B. Prostaglandin synthase-dependent cooxidation and aromatic amine carcinogenesis. In: Arachidonic Acid Metabolism and Tumor Initiation (L. J. Marnett, Ed.), Martinus Nijhoff, Boston, 1985, pp. 125–169.
8. Wells, P. G., Nagai, M. K., and Spano Greco, G. Inhibition of trimethadone and dimethadione teratogenicity by the cyclooxygenase inhibitor acetylsalicylic acid: a unifying hypothesis for the teratologic effects of hydantoin anticonvulsants and structurally related compounds. Toxicol. Appl. Pharmacol. 97: 406–414 (1989).
9. Lands, W. E. M., and Samuelsson, B. Phospholipid precursors of prostaglandins. Biochim. Biophys. Acta 164: 426–429 (1986).
10. Vonkeman, H., and Van Dorp, D. A. The action of prostaglandin synthetase on 2-arachidonyl-lecithin. Biochim. Biophys. Acta 164: 430–432 (1986).
11. Galli, C., Galli, G., and Porcellati, G. Phospholipases and Prostaglandins. Advances in Prostaglandin and Thromboxane Research, Vol. 3. Raven Press, New York, 1978.
12. Roth, G. J., Machuga, E. T., and Ozols, J. Isolation and covalent structure of the aspirin modified, active site region of prostaglandin synthetase. Biochemistry 22: 4672–4675 (1983).
13. Bailey, J. M., Muza, B., Hla, T., and Salata, K. Restoration of prostacyclin synthase in vascular smooth muscle cells after aspirin treatment: regulation by epidermal growth factor. J. Lipid Res. 26: 54–61 (1985).
14. Casey, M. L., Korte, K., and MacDonald, P. C. Epidermal growth factor stimulation of prostaglandin E_2 biosynthesis in amnion cells. Induction of prostaglandin H_2 synthase. J. Biol. Chem. 263: 7846–7854 (1988).
15. Raz, A., Wyche, A., Siegel, N., and Needleman, P. Regulation of fibroblast cyclooxygenase synthesis by interleukin-1. J. Biol. Chem. 263: 3022–3028 (1988).
16. Pfeilschifter, J., Pignat, W., Vosbeck, K., and Marki, F. Interleukin 1 and tumor necrosis factor synergistically stimulate prostaglandin synthesis and phospholipase A_2 release from rat renal mesangial cells. Biochem. Biophys. Res. Comm. 59: 385–394 (1989).
17. Wu, K. K., Hatzakis, H., Lo, S. S., Seong, D. C., Sanduja, S. K., and Tai, H. H. Stimulation of de novo synthesis of prostaglandin G/H synthase in human endothelial cells by phorbol ester. J. Biol. Chem. 263: 19043–19047 (1988).
18. Bailey, J. M., Makheja, A. N., Pash, J., and Verma, M. Corticosteroids suppress cyclooxygenase messenger RNA levels and prostanoid synthesis in cultured vascular cells. Biochem. Biophys. Res. Comm. 157: 1159–1163 (1988).
19. Raz, A., Wyche, A., and Needleman, P. Temporal and pharmacological division of fibroblast cyclooxygenase expression into transcriptional and translation phases. Proc. Natl. Acad. Sci. USA 86: 1657–1661 (1989).
20. Fagan, J. M., and Goldberg, A. L. Inhibitors of protein and RNA synthesis cause a rapid block in prostaglandin production at the prostaglandin synthase step. Proc. Natl. Acad. Sci. USA 83: 2771–2775 (1986).
21. Marnett, L. J., Wlodawer, P., and Samuelsson, B. Co-oxygenation of organic substrates by the prostaglandin synthetase of sheep vesicular gland. J. Biol. Chem. 250: 8510–8517 (1975).
22. Marnett, L. J. Hydroperoxide-dependent oxidations during prostaglandin biosynthesis. In: Free Radicals in Biology, Vol. 6 (W. A. Pryor, Ed.), Academic Press, New York, 1984, pp. 63–94.
23. Hamberg, M., and Samuelsson, B. Oxygenation of unsaturated fatty acids by the vesicular gland of sheep. J. Biol. Chem. 242: 5344–5354 (1967).
24. Spalding, J. W. Toxicology and carcinogenesis studies of malondialdehyde sodium salt (3-hydroxy-2-propenal, sodium salt) in F344/N rats and B6C3F1 mice. NTP Technical Report No. 331. National Toxicology Program, Research Triangle Park, NC, 1989, pp. 5–13.
25. Basu, A. K., and Marnett, L. J. Unequivocal demonstration that malondialdehyde is a mutagen. Carcinogenesis 4: 331–333 (1983).
26. Furstenberger, G., Gross, M., and Marks, F. Eicosanoids and multistage carcinogenesis in NMRI mouse skin: role of prostaglandins E and F in conversion (first stage of tumor promotion) and promotion (second stage of tumor promotion). Carcinogenesis 10: 91–96 (1989).
27. Christ, E. J., and Van Dorp, D. A. Comparative aspects of pros-

taglandin biosynthesis in animal tissue. Biochim. Biophys. Acta 270: 537–541 (1972).
28. Rollins, T. E., and Smith, W. L. Subcellular localization of prostaglandin-forming cyclooxygenase in Swiss mouse 3T3 fibroblasts by electron microscopic immunocytochemistry. J. Biol. Chem. 255: 4872–4875 (1980).
29. Smith, W. L., DeWitt, D. L., and Allen, M. A. Distribution of prostaglandin I_2 synthase antigen in smooth muscle cells. J. Biol. Chem. 258: 5922–5926 (1983).
30. Miyamoto, T., Ogino, N., Yamamoto, S., and Hayaishi, O. Purification of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. J. Biol. Chem. 251: 2629–2636 (1976).
31. van der Ouderaa, F. J., Buytenhek, M., Nugteren, D. H., and Van Dorp, D. A. Purification and characterization of prostaglandin synthetase from sheep vesicular glands. Biochim. Biophys. Acta 487: 315–331 (1977).
32. Hemler, M., Lands, W. E. M., and Smith, W. L. Purification of the cyclooxygenase that forms prostaglandins. Demonstration of two forms of iron in the holoenzyme. J. Biol. Chem. 251: 5575–5579 (1976).
33. Yamamoto, S. Purification and assay of PGH synthase from bovine seminal vesicles. Methods Enzymol. 86: 55–60 (1982).
34. van der Ouderaa, F. J. G., and Buytenhek, M. Purification of PGH synthase from sheep vesicular glands. Methods Enzymol. 86: 60–68 (1982).
35. Kulmacz, R. J., and Lands, W. E. M. Cyclooxygenase: measurement, purification, and properties. In: Prostaglandins and Related Substances: A Practical Approach (C. Benedetto, R. G. McDonald-Gibson, S. Nigam, and T. F. Slater, Eds.), IRL Press, Washington, DC 1987, pp. 209–227.
36. Roth, G. J., Machuga, E. T., and Strittmatter, P. The heme-binding properties of prostaglandin synthetase from sheep vesicular gland. J. Biol. Chem. 256: 10018–10022 (1981).
37. Marnett, L. J., Siedlik, P. H., Ochs, R. C., Pagels, W. D., Das, M., Honn, K. V., Warnock, R. H., Tainer, B. E., and Eling, T. E. Mechanism of the stimulation of prostaglandin H synthase and prostacyclin synthase by the antithrombotic and antimetastatic agent Nafazatrom. Mol. Pharmacol. 26: 328–335 (1984).
38. Mevkh, A. T., Sud'ina, G. F., Golub, N. B., and Varfolomeev, S. D. Purification of prostaglandin H synthetase and a fluorometric assay for its activity. Anal. Biochem. 150: 91–96 (1985).
39. Mutsaers, J. H. G. M., Van Halbeek, H., Kamerling, J. P., and Vliegenthart, J. F. G. Determination of the structure of the carbohydrate chains of prostaglandin endoperoxide synthase from sheep. Eur. J. Biochem. 147: 569–574 (1985).
40. Ogino, N., Ohki, S., Yamamoto, S., and Hayaishi, O. Prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. Inactivation and activation by heme and other metalloporphyrins. J. Biol. Chem. 253: 5061–5068 (1978).
41. Ruf, H. H., Schuhn, D., and Nastainczyk, W. EPR titration of ovine prostaglandin H synthase with hemin. FEBS Let. 165: 293–296 (1984).
42. Kulmacz, R. J., and Lands, W. E. M. Prostaglandin H synthase: stoichiometry of heme cofactor. J. Biol. Chem. 259: 6358–6363 (1984).
43. Merlie, J. P., Fagan, D., Mudd, J., and Needleman, P. Isolation and characterization of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxide synthase (cyclooxygenase). J. Biol. Chem. 263: 3550–3553 (1988).
44. DeWitt, D. L., and Smith, W. L. Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. Proc. Natl. Acad. Sci. USA 85: 1412–1416 (1988).
45. Yokoyama, C., Takai, T., and Tanabe, T. Primary structure of sheep prostaglandin endoperoxide synthase deduced from cDNA sequence. FEBS Let. 231: 347–351 (1988).
46. Marnett, L. J., Chen, Y.-N, P., Maddipati, K. R., Plé, P., and Labeque, R. Functional differentiation of cyclooxygenase and peroxidase activities of prostaglandin synthase by trypsin treatment: possible location of a prosthetic heme binding site. J. Biol. Chem. 263: 16532–16535 (1988).
47. Lambeir, A. M., Markey, C. M., Dunford, H. B., and Marnett, L. J. Spectral properties of the higher oxidation states of prostaglandin H synthase. J. Biol. Chem. 260: 14894–14896 (1985).
48. Karthein, R., Dietz, R., Nastainczyk, W., and Ruf, H. H. Higher oxidation states of prostaglandin H synthase. EPR study of a transient tyrosyl radical in the enzyme during the peroxidase reaction. Eur. J. Biochem. 171: 313–320 (1988).
49. Dunford, H. B., and Stillman, J. S. On the function and mechanism of action of peroxidases. Coord. Chem. Rev. 19: 187–251 (1976).
50. Egan, R. W., Gale, P. H., Vandenheuvel, W. J., Baptista, E. M., and Kuehl, F. A. Mechanism of oxygen transfer by prostaglandin hydroperoxidase. J. Biol. Chem. 255: 323–326 (1980).
51. Egan, R. W., Gale, P. H., Baptista, E. M., Kennicott, K. L., Vandenheuvel, W. J., Walker, R. W., Fargeness, P. E., and Kuehl, F. A. Oxidation reactions by prostaglandin cyclooxygenase hydroperoxidase. J. Biol. Chem. 256: 7352–7361 (1981).
52. Saunders, B. C. Peroxidases and catalases. In: Inorganic Biochemistry (G. L. Eichhorn, Ed.), Elsevier, Amsterdam, 1975, pp. 988–1021.
53. Marnett, L. J., Bienkowski, M. J., Pagels, W. R., and Reed, G. A. Mechanism of xenobiotic cooxygenation coupled to prostaglandin H_2 biosynthesis. In: Advances in Prostaglandin and Thromboxane Research, Vol. 6 (B. Samuelsson, P. W. Ramwell, and R. Paoletti, Eds.), Raven Press, New York, 1980, pp. 149–151.
54. Samokyszyn, V. M., and Marnett, L. J. Hydroperoxide-dependent cooxidation of 13-cis-retinoic acid by prostaglandin H synthase. J. Biol. Chem. 262: 14119–14133 (1987).
55. Hughes, M. F., Mason, R. P., and Eling, T. E. Prostaglandin hydroperoxidase-dependent oxidation of phenylbutazone: relationship to inhibition of prostaglandin cyclooxygenase. Mol. Pharmacol. 34: 186–193 (1988).
56. Eling, T. E., Mason, R. P., and Sivarajah, K. The formation of aminopyrine cation radical by the peroxidase activity of prostaglandin H synthase and subsequent reactions of the radical. J. Biol. Chem. 260: 1601–1607 (1985).
57. Josephy, P. D., and Iwaniw, D. C. Identification of the N-acetylcysteine conjugate of benzidine formed in the peroxidase activation system. Carcinogenesis 6: 155–158 (1985).
58. Bull, A. W. Reducing substrate activity of some aromatic amines for prostaglandin H synthase. Carcinogenesis 8: 387–390 (1987).
59. Robertson, I. G. C., Sivarajah, K., Eling, T. E., and Zeiger, E. Activation of some aromatic amines to mutagenic products by prostaglandin endoperoxide synthetase. Cancer Res. 43: 476–480 (1983).
60. Wild, D., and Degen, G. H. Prostaglandin H synthase-dependent mutagenic activation of heterocyclic aromatic amines of the IQ-type. Carcinogenesis 8: 541–545 (1987).
61. Degen, G. J., Wong, A., Eling, T., Barrett, J. C., and McLachlan, J. A. Involvement of prostaglandin synthetase in the peroxidative metabolism of diethylstilbestrol in Syrian hamster embryo fibroblast cell cultures. Cancer Res. 43: 992–996 (1983).
62. Ingold, K. Peroxy radicals. Acc. Chem. Res. 2: 1–9 (1969).
63. Bollag, W. Prophylaxis of chemically induced benign and malignant epithelial tumors by vitamin A (retinoic acid). Eur. J. Cancer 8: 689–693 (1972).
64. Verma, A. K., Rice, H. M., Shapas, B. G., and Boutwell, R. K. Inhibition of 12-O-tetradecanoyl-phorbol-13-acetate-induced ornithine decarboxylase activity in mouse epidermis by vitamin A analogs (retinoids). Cancer Res. 38: 793–801 (1978).
65. Roberts, A. B., and Sporn, M. B. Cellular biology and biochemistry of the retinoid. In: The Retinoids (M. B. Sporn, A. B. Roberts, and D. S. Goodman, Eds.), Academic Press, New York, 1984, pp. 210–286.
66. Samokyszyn, V. M., and Marnett, L. J. Inhibition of liver microsomal lipid peroxidation by 13-cis-retinoic acid. Free Radic. Biol. Med., in press.
67. Dix, T. A., and Marnett, L. J. Conversion of linoleic acid hydroperoxide to hydroxy, keto epoxyhydroxy, and trihydroxy fatty acids by hematin. J. Biol. Chem. 260: 5351–5367 (1985).
68. Dix, T. A., Fontana, R., Panthani, A., and Marnett, L. J. Hematin-catalyzed epoxidation of 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (BP-7,8-diol) by polyunsaturated fatty acid hydroperoxides. J. Biol. Chem. 260: 5358–5365 (1985).

69. Porter, N. A. Mechanisms for the autoxidation of polyunsatured lipids. Acc. Chem. Res. 19: 262–268 (1986).
70. Marnett, L. J. Peroxyl free radicals: potential mediators of tumor initiation and promotion. Carcinogenesis 8: 1365–1373 (1987).
71. Pryor, W. A. Oxy-radicals and related species: their formation, lifetimes and reactions. Ann. Rev. Physiol. 48: 657–667 (1986).
72. Mayo, F. R. Free-radical autoxidations of hydrocarbons. Acc. Chem. Res. 1: 193–201 (1968).
73. Marnett, L. J., Reed, G. A., and Dennison, D. J. Prostaglandin synthetase dependent activation of 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene to mutagenic derivatives. Biochem. Biophys. Res. Comm. 82: 210–216 (1978).
74. Marnett, L. J., Johnson, J. T., and Bienkowski, M. J. Arachidonic acid-dependent metabolism of 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene by ram seminal vesicles. FEBS Lett. 106: 13–16 (1979).
75. Sivarajah, K., Mukhtar, H., and Eling, T. E. Arachidonic acid-dependent metabolism of (±)-*trans*-7,8-dihydroxy-7,8-dihyrobenzo[a]pyrene (BP-7,8-diol) to 7,10/8,9-tetrols. FEBS Lett. 106: 17–20 (1979).
76. Dix, T. A., Buck, J. R., and Marnett, L. J. Hydroperoxide-dependent epoxidation of 3,4-dihydroxy-3,4-dihydrobenzo[a]-anthracene by ram seminal vesicle microsomes and by hematin. Biochem. Biophys. Res. Commun. 140: 181–187 (1986).
77. Battista, J. R., and Marnett, L. J. Prostaglandin H synthase-dependent epoxidation of aflatoxin B_1. Carcinogenesis 6: 1227–1229 (1985).
78. Dix, T. A., and Marnett, L. J. Metabolism of polycyclic aromatic hydrocarbon derivative to ultimate carcinogens during lipid-peroxidation. Science 221: 77–79 (1983).
79. Battista, J. R., Dix, T. A., and Marnett, L. J. The mechanism of hydroperoxide-dependent epoxidation of 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene by rat liver microsomes (abstract). Proc. Am. Assoc. Cancer Res. 25: 114 (1984).
80. Reed, G. A., Brooks, E. A., and Eling, T. E. Phenylbutazone-dependent epoxidation of 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene: a new mechanism for prostaglandin H synthase-catalyzed oxidations. J. Biol. Chem. 259: 5591–5595 (1984).
81. Reed, G. A., Curtis, J. F., Mottley, C., Eling, T. E., and Mason, R. P. Epoxidation of (±)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene during (bi)sulfite autoxidation: activation of a procarcinogen by a cocarcinogen. Proc. Natl. Acad. Sci. USA 83: 7499–7502 (1986).
82. Krauss, R. S., and Eling, T. E. Formation of unique arylamine: DNA adducts from 2-aminofluorene activated by prostaglandin H synthase. Cancer Res. 45: 1680–1686 (1985).
83. Eling, T. E., and Kadlubar, F. F. DNA adducts formed from β-naphthylamine in dog urinary bladder. J. Biochem. Toxicol. 4: 111–117 (1989).
84. Panthananickal, A., and Marnett, L. J. Arachidonic acid-dependent metabolism of (±)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene to polyguanylic acid-binding derivatives. Chem.-Biol. Interact. 33: 239–252 (1981).
85. Dix, T. A., and Marnett, L. J. Detection of the metabolism of polycyclic aromatic hydrocarbon derivatives to ultimate carcinogens during lipid peroxidation. Methods Enzymol. 105: 347–352 (1984).
86. Pruess-Schwartz, D., Nimesheim, A., and Marnett, L. J. Peroxyl radical-and cytochrome P-450-dependent metabolic activation of (+)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene in mouse skin in vitro and in vivo. Cancer Res. 49: 1732–1737 (1989).
87. Boyd, J. A., Barrett, J. C., and Eling, T. E. Prostaglandin endoperoxide synthetase dependent cooxidation of (±)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene in C3H10T1/2 clone 8 cells. Cancer Res. 42: 2828–2832 (1982).
88. Reed, G. A., Grafstrom, R. C., Krauss, R. S., Autrup, H., and Eling, T. E. Prostaglandin H synthase-dependent co-oxygenation of (±)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene in hamster trachea and human bronchus explants. Carcinogenesis 5: 955–960 (1984).
89. Eling, T., Curtis, J., Battista, J., and Marnett, L. J. Oxidation of (±)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene by mouse keratinocytes: evidence for peroxyl radical- and monoxygenase-dependent metabolism. Carcinogenesis 7: 1957–1963 (1986).
90. Melikian, A. A., Bagheri, K., and Hecht, S. S. Contrasting disposition and metabolism of topically applied benzo[a]pyrene, *trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene, and 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene in mouse skin epidermis in vivo. Cancer Res. 47: 5354–5360 (1987).
91. Robinson, H. J., and Vane, J. R. Prostaglandin Synthase Inhibitors—Their Effects on Physiological Functions and Pathological States. Raven Press, New York, 1974.